

ON THE CHROMOGEN AND ON THE ACTIVE PHYSIOLOGICAL SUBSTANCE OF THE SUPRARENAL GLAND. BY B. MOORE.

(From the Physiological Laboratory of University College, London.)

IN a preliminary communication made to the Physiological Society in March 1895¹, I gave some account of the chemical and physical properties of the substance occurring in the medulla of the suprarenal gland, the remarkable physiological action of which was first described by Schäfer and Oliver a year previously².

These reactions did not enable me to identify the substance from the chemical standpoint, but they led me to the conclusion that it was identical with a powerfully reducing substance first described by Vulpian³ in the same situation. This material gives various colour reactions on being oxidised; *e.g.* with ferric chloride it gives a dark green or blue colour, and with chlorine, bromine, or iodine water or caustic alkalies a rose-red colour.

Since then a paper has appeared by S. Fränckel⁴, who agrees with my surmise that the chromogen is identical with the active physiological substance in the gland; he further claims to have isolated and advanced a step towards the identification of the active material.

His method of isolating the material depends upon its solubility in absolute alcohol.

His process is as follows:—

Watery or alcoholic extracts of suprarenals were evaporated to a syrup. If alcoholic extracts were used the residue was dissolved in water, if water extracts then the residue was dissolved in alcohol. After filtering and again evaporating, the residue was taken up with boiling absolute alcohol, and after

¹ *Proc. Physiol. Soc.* p. xiv (This Journal, xvii. 1895).

² *Proc. Physiol. Soc.* p. i (This Journal, xvi. 1894).

³ *Comptes Rendus*, XLIII. 1856; XLV. 1857. See also Virchow, *Virchow's Archiv*, xii. 1857; Arnold, *Ibid.* xxxv. 1866; Kruckenberg. *Ibid.* ci. 1885; Brunner, *Chem. Centralb.* 1892, i. p. 758.

⁴ *Wiener Mediz. Blätter*, 1896, Nr. 14—16.

cooling and filtering an equal volume of acetone was added to the alcoholic solution. The only impurities which could be removed in this way are those which are soluble in alcohol and insoluble in a mixture of alcohol and acetone; it is very improbable that these are the only impurities present. This caused a crystalline precipitate of an organic substance mixed with potassium chloride. The alcohol-acetone solution was repeatedly evaporated down, which caused further separations of crystalline substance; the syrup was dissolved in alcohol and precipitated by ether. Finally, a syrupy substance was obtained which he was unable to obtain in a crystalline form, but a trace of which showed the characteristic influence on the blood-pressure.

This syrupy material is regarded by Fränckel as a pure substance, but no chemical criteria are given of its purity. It is not shown that it possesses a constant percentage composition, and no attempt is made to establish even an empirical formula for it.

My own experiments had shown me that the active substance of the gland is almost insoluble in absolute alcohol, and that its activity is destroyed by repeated treatment with that reagent, especially if heated in the process. It was therefore surprising to me that Fränckel should have obtained a material a trace of which produced a large rise of blood-pressure, even after repeated treatment with boiling absolute alcohol.

Further experiment has led me to conclude that Fränckel's opinion that the active substance is a derivative of pyrocatechin is incorrect, and also that it is not identical with the chromogen of the organ.

A large number of sheep's suprarenals were taken a few hours after the slaughter of the animals, cleared of fats, split open, and the medulla dissected out accompanied by as little cortex as possible. The medulla was placed under a large excess of strong alcohol (meth. spirit. of 98%) in a closed flask for fourteen days¹. At the end of this time a water extract of a portion of the medulla thus treated, as well as other products obtained from it, was tested physiologically by injection. I was surprised to obtain no trace of an effect, either from the water extract or any of the other products. On testing for the chromogen it was also found to be only present in traces. The alcohol under which the medulla had been standing was next tested for the chromogen and was found to contain it in abundance. This was next tested by injection, and I was again surprised on obtaining not a trace of an effect. The whole experiment was repeated three times with the same result. To make the matter quite certain, a sample of the dried and powdered

¹ About 30 gms. of medulla in 400 c.c. of spirit.

suprarenal glands of the sheep which was known to be very active was taken. Two portions of .5 gram each were weighed out and placed in two small bottles. To one bottle 10 c.c. of spirit were added; in the other the powder was left dry. Both bottles were tightly corked, and after the lapse of a month the spirit in the one was decanted off, and each powder was extracted with 10 c.c. of boiling normal saline.

The two saline extracts after filtering and also the spirit were tested by injection. One c.c. of the extract obtained from the powder which had stood under spirit, or one c.c. of the spirit, produced no trace of an effect on arterial blood-pressure; while $\frac{1}{3}$ c.c. of the extract of the powder which had not been treated with spirit caused a very large rise of blood-pressure. The spirit was finally tested for the chromogen and found to contain abundance of it, giving the usual green with ferric chloride and deep rose-red with caustic potash.

This experiment shows clearly that the substance, or organic radical, which gives the colour tests has no action on blood-pressure. The experiment may mean either that the chromogen has no connection whatever with the active substance, or that the physiological action is due to some group in the molecule which can be destroyed without affecting the chromogen group. The close association of the chromogen and the physiological action and the ease with which both are destroyed by oxidizing agents are rather in favour of the latter view.

Nothing can be affirmed with certainty as to the chemical nature and relationships of either the chromogen or the active substance until these have been satisfactorily isolated and their chemical constitution determined, but in the meantime it may be of some interest and possibly of some assistance to others working on the subject to briefly present certain ideas which have suggested themselves in the course of my experiments.

First with regard to the chromogen. The reaction with ferric chloride and the power of reducing silver nitrate and not Fehling's solution are characteristic of a large number of organic compounds all of which contain an *orthodihydroxy-benzene* group. The members of this class are also like the suprarenal chromogen easily oxidized in alkaline solution.

There are hundreds of such compounds already known in organic chemistry. Where among all these are we to search for the chromogen of the suprarenal? In this quest some assistance is given by the solubilities of the chromogen. Nearly all the *orthodihydroxy-benzene* derivatives are soluble like their mother substance pyrocatechin in

water, alcohol, and ether. The only exceptions to this rule which I have been able to discover are the salts of certain acids (such as protocathechuic acid) containing this group, and a few more complex substances, belonging to the group of the tannic acids. The chromogen does not belong to the first of these classes, for a salt of an organic acid dissolved in water and treated with a mineral acid gives the free acid, which if soluble in ether may be extracted by that solvent. Now although the salts of the organic acids containing an orthodihydroxy-benzene group are insoluble in ether, the free acids are readily soluble and may be removed from solution in water thereby. On so treating a solution of the suprarenal chromogen, this does not pass into ethereal solution.

The suprarenal material more probably belongs to the second class, which do not suffer decomposition with detachment of the orthodihydroxy-benzene group on treatment with dilute mineral acids.

The tannins or tannic acids as a class are soluble in water and insoluble in absolute alcohol or anhydrous ether. They all react with ferric chloride, giving either blue or green colorations. Those giving a green coloration usually contain an orthodihydroxy-benzene group, and on dry distillation or fusion with caustic alkalies yield pyrocatechin or protocathechuic acid. As examples may be mentioned those of coffee and tobacco, each of which gives a green colour with ferric chloride, turning to red on the addition of an alkali.

It is more probable that the suprarenal chromogen is a complex compound somewhat resembling these than that it is a simple derivative of pyrocatechin; the other part of the molecule which does not contain the pyrocatechin radical perhaps being that which confers physiological activity. This active group might easily be attacked by a reagent which left the pyrocatechin group intact; this would explain the loss of activity combined with preservation of chromogen experimentally observed.

That the activity is not due to the pyrocatechin group is further shown by the fact that neither pyrocatechin nor protocathechuic acid produces any appreciable effect on blood-pressure, causing merely an insignificant rise when injected in much greater quantity than suprarenal extract.

Mühlmann¹ claims to have obtained pyrocatechin from suprarenal extract by heating it for 15 minutes with 10 per cent. hydrochloric acid, and after extraction with ether. The substance obtained may

¹ *Deutsche med. Wochensch.* 1896, No. 26.

have been pyrocatechin, but the evidence given in the paper only goes the length of showing that it is soluble in ether and gives the ferric chloride reaction, and these are common to many pyrocatechin derivatives, including the suprarenal chromogen itself unless the ether be dry.

Both Mühlmann and Fränckel argue from slight differences in colour reactions that the suprarenal material is not pyrocatechin itself. This seems to me to be sufficiently demonstrated by the respective solubilities of the two substances, and by the comparatively infinitesimal action of pyrocatechin on blood-pressure. Both authors however consider that the effect of pyrocatechin on blood-pressure indicates that the active material is allied to that body, and Fränckel announces the intention of searching for the body among the synthetic derivatives of pyrocatechin.

Before Fränckel's announcement I had already tried all of these which I could easily procure, especially those which by their solubilities might be expected to have any relationship to the suprarenal chromogen, including salts of protocatechuic acid, and all those tannins which give a green colour with ferric chloride.

None of these gave an effect at all comparable with that of suprarenal extract, usually the blood-pressure was not in the least affected. Taken in conjunction with the effect of prolonged contact with alcohol above described, these experiments have convinced me that the pyrocatechin group has nothing to do with the activity.

No definite results have been hitherto obtained as to the nature of the substance or group on which the physiological activity depends, but there are certain indications that it is probably a pyridine derivative.

If suprarenal extract be dissolved in 50 per cent. alcohol, and basic acetate of lead be cautiously added, a flesh-coloured precipitate is obtained. If this be washed with 50 per cent. alcohol, suspended in more of the same fluid, and decomposed by sulphuretted hydrogen, a pale yellow, usually fluorescent solution is obtained.

This solution raises blood-pressure on injection but not to the same extent as the original extract, and on repetition of the process of precipitation all the active material is finally destroyed. On examining the filtrate from the basic acetate of lead, after removal of excess of lead by sulphuretted hydrogen, it is found that it is totally inactive. This shows that the active material is precipitated by basic acetate of lead, but is destroyed or converted into some inactive product in the process.

The destruction is probably due to the alkaline reaction of the basic acetate of lead, causing oxidation and destruction as do ordinary alkalies. The solution obtained by the decomposition of the lead precipitate when it has ceased to be active therefore probably contains the oxidised wreck of the active substance. When the solution is evaporated to dryness, a reddish-brown mass is obtained, sticky and semi-fluid when hot, and brittle on cooling and very hygroscopic. In all this it closely resembles the original extract. Arnold and Krukenberg had previously attempted but without success to isolate the chromogen of the gland from such a lead precipitate, which they decomposed with oxalic acid in alcoholic solution instead of with sulphuretted hydrogen. Krukenberg however made analyses of a product very similar to that above described. This material was undoubtedly somewhat impure and considering its hygroscopic character contained more or less water. Krukenberg does not attempt to construct a formula from his analysis but his results agree fairly closely, for such a substance, with the formula $C_5H_{14}NO_4$, as may be seen by a comparison of the following figures.

Krukenberg's results.	Calculated for formula $C_5H_{14}NO_4$.
Carbon 40·83 %	Carbon 39·5 %
Hydrogen 9·1 %	Hydrogen 9·2 %
Nitrogen 9·05 %	Nitrogen 9·2 %
Oxygen (diff.) 41·02 %	Oxygen 42·1 %

When it is remembered that these results are obtained from a hygroscopic non-crystallizable material, the agreement is as close as can be expected. The point of most interest is the relationship between carbon and nitrogen in the formula, five atoms of the former to one of the latter. Now this is the ratio in which these elements are found in pyridene (C_5H_6N), the mother substance of all or nearly all the numerous class of the vegetable alkaloids¹, and it is in just such a class of compounds, all of which possess marked physiological actions, that a body such as the active material might be expected to be found.

The above result having suggested that the active material might be a pyridene derivative, some suprarenal extract was cautiously fused with caustic potash so as to avoid charring, when the peculiar odour of pyridene was at once obtained. Of course pyridene and its homo-

¹ See A. Pictet, *Die Pflanzenalkalöide und ihre chemische Konstitution*, German edition, 1891, S. 5.

logues are formed in the destructive distillation of proteids, so that one must be careful in concluding from this experiment that a pyridene derivative is present; however, the experiment was conducted so as to avoid charring, and taken in conjunction with the above result makes it probable that suprarenal extract contains a pyridene derivative.

That the physiological action is due to this pyridene derivative is rendered probable not only by the behaviour of the lead precipitate described above in finally yielding the oxidized residue devoid of action, but by the fact that all the pyridene compounds are physiologically active, the character of the action varying with the molecular grouping in the molecule of the particular derivative, and so giving origin to the varied physiological action of the different alkaloids.

The action of the different pyridene derivatives on blood-pressure is widely different. Pyridene itself, as has recently been shown by Lauder Brunton, causes a marked depression of blood-pressure when intravenously injected, while nicotine is well known to produce a prodigious rise even in minute doses. Now nicotine contains two pyridene rings, in one of which the pyridene is reduced by the introduction of six hydrogen atoms to piperidine, that is, nicotine is formed by the union of a molecule of pyridene with a molecule of reduced pyridene, or piperidine. Since the pyridene molecule causes a marked fall in blood-pressure it struck me that the immense rise caused by nicotine might be due to the reduced pyridene or piperidine ring. I accordingly injected intravenously a small dose of piperidine (10—15 milligrams in a dog of 4·5 kg.) and found my surmise verified by the result, the carotid blood-pressure was more than trebled. At this time I was unaware that the effects of injection of piperidine on blood-pressure were known, but I soon after found out that I had been preceded by Tunnicliffe¹ who describes a precisely similar effect, which he states persists after section of the cord at the occipito-atlantoid foramen. I find that the free base causes powerful constriction of the arterioles when perfused through the vessels of a pithed frog in a concentration of 1 in 1000 of normal saline (·75 per cent.), but when neutralized with acetic acid has no such effect, showing that the peripheral constricting action of the free base is probably due to the alkalinity, and that the effect on intravenous injection which is obtained also with neutralized solutions is probably more central in origin.

¹ Tunnicliffe, *Centralblatt f. Physiol.* March 8th, 1897.

This change in passing from pyridene to piperidine is an interesting case of connection between chemical constitution and physiological action.

The alkaloid of pepper piperin contains in its molecule a molecule of piperidin united to a molecule of an organic acid (piperic acid). This body also produces a marked rise in arterial blood-pressure on intravenous injection, probably again due to the presence of the reduced pyridene molecule.

Although the rise of blood-pressure caused by this class of substances closely resembles in extent and form of the curve that caused by suprarenal material yet the action is not the same. Suprarenal acts peripherally, these drugs most probably resemble nicotine and act by stimulating nerve-cells. On the other hand, both suprarenal material and piperidine have their activity destroyed by oxidation, and probably the suprarenal material like piperidine is a pyridene derivative, which contains a reduced or partially reduced pyridene ring, differing somewhat from piperidine.